

What is claimed is:

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5 1. A method of conferring resistance to protoporphyrinogen oxidase-inhibiting herbicides upon plants or plant cells, comprising introducing a DNA fragment, or biologically functional equivalent thereof, or a plasmid containing the DNA fragment or its biological equivalent, into plants or plant cells, wherein said DNA fragment or said biologically functional equivalent is expressed and has the following characteristics:

(1) said DNA fragment encodes a protein or a part of the protein having protoporphyrinogen activity in plants;

15 (2) said DNA fragment is homologous to a nucleic acid encoding an amino acid sequence selected from the group consisting of SEQ. ID. NO.: 1, SEQ. ID. NO.: 2 or SEQ. ID. NO.: 3, and encodes a protein or part of a protein in which an amino acid corresponding to Val13 of SEQ. ID. NO.: 1 or SEQ. ID. No.: 2 or SEQ. ID. No.: 3 is substituted by another amino acid; that can be detected and isolated by DNA-DNA or DNA-RNA hybridization methods; and

20 (3) said DNA fragment has an ability to confer resistance to protoporphyrinogen oxidase-inhibiting herbicides in plant or algal cells when expressed therein.

25

5 *SK*
2. The method according to claim 1, wherein the DNA fragment or biologically functional equivalent thereof, or a plasmid containing the DNA fragment encodes a protein or a part of the protein having protoporphyrinogen oxidase activity in a dicot.

3. The method according to claim 2, wherein the dicot is *Arabidopsis thaliana*, and the DNA fragment encodes a protein in which Val13 of SEQ. ID. NO.: 2 is substituted with another amino acid.

10 *SK*
4. The method according to claim 1, wherein the DNA fragment encodes a protein or a part of the protein having protoporphyrinogen oxidase activity in a monocot.

15 5. The method according to claim 4, wherein the DNA fragment encodes a protein or a part of the protein having protoporphyrinogen oxidase activity in maize, and the DNA fragment encodes a protein in which Val13 of SEQ. ID. NO.: 3 is replaced by another amino acid.

20 *SK*
6. The method according to claim 1, wherein the DNA fragment encodes a protein or a part of the protein having protoporphyrinogen oxidase activity in *Chlamydomonas*, and the DNA fragment encodes a protein in which Val13 of SEQ. ID. NO.: 1 is replaced by another amino acid.

25 7. The method according to any one of claims 1 to 6, wherein Val13 or the corresponding amino acid is replaced by methionine.

30 8. The method according to any one of claims 1 to 6, wherein the plant or plant cells upon which resistance is conferred is the green alga

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Chlamydomonas

9. The method of conferring resistance to protoporphyrinogen-inhibiting herbicides according to claim 8, wherein Val13 of the corresponding amino acid is replaced by methionine.

10. A plant or plant cells or green alga upon which resistance is conferred by the method described in ~~any one of claims 1 to 9.~~ *claim 8*

11. A method of selecting plant or algal cells upon which resistance to protoporphyrinogen-inhibiting herbicides is conferred, which comprises treating a population of plant or algal cells, upon which resistance to protoporphyrinogen-inhibiting herbicides is conferred by the method as described in ~~any one of claims 1 to 9,~~ *claim 8* with a protoporphyrinogen-inhibiting herbicide in an amount which normally blocks growth of said plant or algal cells expressing only herbicide-sensitive protoporphyrinogen oxidase.

12. A method of controlling plants lacking resistance to protoporphyrinogen-inhibiting herbicides in cultivated fields of crop plants upon which resistance to protoporphyrinogen-inhibiting herbicides is conferred by the method as described in ~~any one of claims 1 to 9~~ *claim 8* which comprises applying to said field at least one protoporphyrinogen-inhibiting herbicide in effective amounts to inhibit growth of said plants lacking resistance to protoporphyrinogen-inhibiting herbicides.

13. The method of controlling non-resistant plants according to claim 12, wherein the protoporphyrinogen-inhibiting herbicides to be applied

are selected from the group of compounds of the formula X - Q, wherein Q is selected from the group consisting of:



(Formula 1)



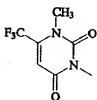
(Formula 2)



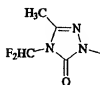
(Formula 3)



(Formula 4)



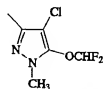
(Formula 5)



(Formula 6)



(Formula 7)



(Formula 8)



(Formula 9)

and



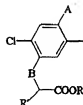
(Formula 10)

and X is selected from the group consisting of



(Formula 11)

wherein
A = H, halogen
B = O, S
R = C₁-C₈ alkyl,
C₃-C₈ alkenyl,
C₃-C₈ alkynyl



(Formula 12)

wherein
A = H, halogen
B = O, S
R' = H, CH₃
R = C₁-C₈ alkyl,
C₃-C₈ alkenyl,
C₃-C₈ alkynyl



(Formula 13)

wherein
A = H, halogen
R = C₁-C₈ alkyl,
C₃-C₈ alkenyl,
C₃-C₈ alkynyl



(Formula 14)

wherein
A = H, halogen
B = O, S
R = C₁-C₈ alkyl,
C₃-C₈ alkenyl,
C₃-C₈ alkynyl



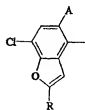
(Formula 15)

wherein
A = H, halogen
R = C₁-C₈ alkyl,
C₃-C₈ alkenyl,
C₃-C₈ alkynyl



(Formula 16)

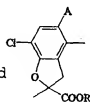
wherein
A = H, halogen
R = C₁-C₈ alkyl,
C₃-C₈ alkenyl,
C₃-C₈ alkynyl



(Formula 17)

wherein
A = H, halogen
R = C₁-C₈ alkyl,
C₃-C₈ alkenyl,
C₃-C₈ alkynyl

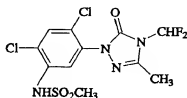
and



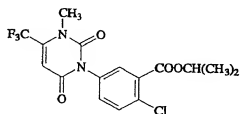
(Formula 18)

wherein
A = H, halogen
R = C₁-C₈ alkyl,
C₃-C₈ alkenyl,
C₃-C₈ alkynyl

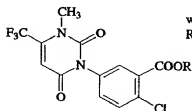
14. The method of controlling non-resistant plants according to claim 12, wherein the protoporphyrinogen-inhibiting herbicide to be applied is selected from the group consisting of compounds of the formula:



(Formula 19)

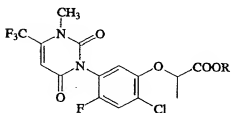


(Formula 20)



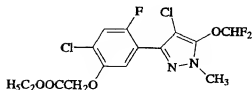
(Formula 21)

wherein
R = (C₂-C₅ alkenyloxy) C₁-C₄ alkyl



(Formula 22)

wherein
R = C₁-C₈ alkyl,
C₃-C₈ alkenyl,
C₃-C₈ alkynyl



(Formula 23)

lactofen,

[N-(4-chloro-2-fluoro-5-propargyloxy)phenyl-3,4,5,6-tetrahydrophthalimide,

5 pentyl [2-chloro-5-(cyclohex-1-ene-1,2-dicarboximido)-4-fluorophenoxy] acetate,

7-fluoro-6-[(3,4,5,6-tetrahydro)phthalimido]-4-(2-propynyl)-1,4-benzoxazin-3(2H)-one,

6-[(3,4,5,6-tetrahydro)phthalimido]-4-(2-propynyl)-1,4-benzoxazin-3(2H)-one,

10 2-[7-fluoro-3-oxo-4-(2-propynyl)-3,4-dihydro-2H-1,4-benzoxazin-6-yl]perhydroimidazo[1,5-a]pyridine-1,3-dione,

2-[(4-chloro-2-fluoro-5-propargyloxy)phenyl] perhydro-1H-1,2,4-triazolo-[1,2-a]pyridazine-1,3-dione,

15 2-[7-fluoro-3-oxo-4-(2-propynyl)-3,4-dihydro-2H-1,4-benzoxazin-6-yl]5,6,7,8-1,2,4-triazolo[4,3-a]pyridine-3H-one,

20 2-[3-oxo-4-(2-propynyl)-3,4-dihydro-2H-1,4-benzoxazin-6-yl]-1-methyl-6-trifluoromethyl-2,4(1H,3H)-pyrimidinedione,

2-[6-fluoro-2-oxo-3-(2-propynyl)-2,3-dihydrobenzthiazol-5-yl]-3,4,5,6-tetrahydrophthalimide, and

25 1-amino-2-[3-oxo-4-(2-propynyl)-3,4-dihydro-2H-1,4-benzoxazin-6-yl]-6-tri-fluoromethyl-2,4(1H,3H)-pyrimidinedione.

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15. A DNA fragment or biologically functional equivalent thereof which has following characteristics:

5 (1) said DNA fragment encodes a protein or a part of the protein having protoporphyrinogen oxidase activity in plants;

(2) said DNA fragment has a sequence that can be detected and isolated by DNA-DNA or DNA-RNA hybridization to a nucleic acid sequence homologous to a nucleic acid sequence encoding an amino acid sequence selected from the group consisting of SEQ. ID. No.: 1, SEQ. ID. No.: 2 and SEQ. ID. No.: 3;

10 (3) said DNA fragment encodes a protein in which an amino acid corresponding to Val13 of SEQ. ID. No.: 1, SEQ. ID. No.: 2 or SEQ. ID. No.: 3 is substituted by another amino acid; and

15 (4) said DNA fragment has the ability to confer resistance to protoporphyrinogen-inhibiting herbicides in plant or algal cells when expressed therein.

20 16. The DNA fragment or biologically functional equivalent thereof according to claim 15, wherein the DNA fragment encodes a protein or a part of the protein having protoporphyrinogen oxidase activity in a dicot.

25 17. The DNA fragment or biologically functional equivalent thereof according to claim 16, wherein the dicot is *Arabidopsis thaliana* and the DNA fragment encodes an amino acid sequence resulting from the replacement of Val13 of SEQ. ID. NO.: 2 by another amino acid.

30 18. The DNA fragment or biologically functional equivalent thereof according to claim 15, wherein the plant is a monocot.

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19. The DNA fragment or biologically functional equivalent thereof according to claim 18, wherein the monocot is maize and the DNA fragment encodes an amino acid sequence resulting from replacement of Vall3 of SEQ. ID. NO.: 3 by another amino acid.

20. The DNA fragment or biologically functional equivalent thereof according to claim 15, wherein the plant is the green alga *Chlamydomonas* and the DNA fragment encodes an amino acid sequence resulting from replacement of Vall3 of SEQ. ID. NO.: 1 by another amino acid.

21. The DNA fragment or biologically functional equivalent thereof according to any one of claims 15 to 20, wherein said another amino acid is methionine.

22. The DNA fragment or biologically functional equivalent thereof according to claim 20, wherein the DNA fragment has a sequence that can be isolated from genomic DNA of *Chlamydomonas* and encodes a protein or a part of the protein having protoporphyrinogen oxidase activity, and a nucleotide corresponding to guanine at position 37 (G37) of SEQ. ID. NO.: 4 is replaced with another nucleotide.

23. The DNA fragment or biologically functional equivalent thereof according to claim 22, wherein said another nucleotide is adenine.

24. A plasmid comprising the DNA fragment or biologically functional equivalent thereof described in ~~in any one of claims 15 to 23.~~ ^{claim 15}

25. A microorganism harboring the plasmid described in claim 24.

26. A method of evaluating the inhibitory effect of a compound on protoporphyrinogen oxidase, comprising (a) culturing in the presence of a test compound a sensitive microorganism containing a gene encoding a protein with protoporphyrinogen oxidase activity sensitive to protoporphyrinogen inhibitors and a resistant microorganism which differs from said sensitive microorganism only by a gene encoding a protein with protoporphyrinogen oxidase activity resistant to protoporphyrinogen inhibitors in which the amino acid corresponding to Val13 of SEQ. ID. No.: 1, SEQ. ID. No.: 2 or SEQ. ID. No.: 3 is replaced with another amino acid and (b) measuring the growth of both of said sensitive and resistant microorganisms to evaluate the inhibitory effect of the test compounds on protoporphyrinogen oxidase.

27. The method of evaluating the protoporphyrinogen oxidase-inhibitory effect according to claim 26, wherein the resistant microorganism is obtained by introducing a gene encoding a protein having protoporphyrinogen oxidase activity resistant to porphyrin herbicides in which the Val13 of SEQ. ID. No.: 1, SEQ. ID. No.: 2 or SEQ. ID. No.: 3 is replaced by another amino acid in a microorganism lacking active protoporphyrinogen oxidase, thereby restoring the growth ability of the microorganism.

28. The method of evaluating the protoporphyrinogen oxidase-inhibitory effect according to claim 26, wherein the resistant microorganism is obtained by introducing a resistant gene encoding a protein having protoporphyrinogen oxidase activity, in which the Val13 of SEQ. ID. No.: 1, SEQ. ID. No.: 2 or SEQ. ID. No.: 3 is replaced by another amino acid, into a *Chlamydomonas* strain sensitive to protoporphyrinogen oxidase-inhibiting herbicides.

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29. A method of evaluating the protoporphyrinogen oxidase-inhibitory effect according to claim 26, wherein the gene that can confer resistance is a gene comprising a DNA fragment as described in claim 20 ~~or 22~~.

30. The method of evaluating the inhibitory effect on protoporphyrinogen oxidase as claimed in any one of claims 26 to 29, wherein Val13 is replaced by methionine or G37 is replaced by adenine, respectively.

31. An *in vivo* method of identifying and evaluating protoporphyrinogen oxidase inhibitors, comprising (a) culturing in the presence of a test compound a sensitive microorganism having a gene encoding a protein with protoporphyrinogen oxidase activity sensitive to a protoporphyrinogen oxidase inhibitor and a resistant microorganism differing from said sensitive microorganism only by the presence of a gene encoding a protein with protoporphyrinogen oxidase activity resistant to a protoporphyrinogen oxidase inhibitor in which an amino acid corresponding to Val13 of SEQ. ID. No.: 1, SEQ. ID. No.: 2 or SEQ. ID. No.: 3 is replaced by another amino acid, and (b) identifying the compound which inhibits growth of only the sensitive microorganism at a particular dosage.

32. The method of selecting a protoporphyrinogen inhibitor according to claim 31, wherein the resistant microorganism is obtained by introducing a gene encoding a protein having protoporphyrinogen oxidase activity resistant to porphyrin herbicides, in which the Val13 of SEQ. ID. No.: 1, SEQ. ID. No.: 2 or SEQ. ID. No.: 3 is replaced by another amino acid, into a microorganism lacking active protoporphyrinogen oxidase, thereby restoring the growth ability of the

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microorganism.

33. The method of selecting a protoporphyrinogen
oxidase inhibitor according to claim 31, wherein the
resistant microorganism is obtained by introducing a
gene encoding a protein having protoporphyrinogen
oxidase activity, in which the Val13 of SEQ. ID. No.:
1, SEQ. ID. No.: 2 or SEQ. ID. No.: 3 is replaced by
another amino acid, into a *Chlamydomonas* strain
sensitive to protoporphyrinogen oxidase-inhibiting
herbicides.

34. The method of selecting a protoporphyrinogen
oxidase inhibitor according to claim 31, wherein said
gene encoding a protein with protoporphyrinogen
oxidase activity resistant to the protoporphyrinogen
oxidase inhibitor is a gene comprising a DNA fragment
as claimed in ~~either of claims 20 or 22.~~ *claim 30*

35. The method of selecting a protoporphyrinogen
oxidase inhibitor according to ~~any one of claims 31 to~~ *claim 31*
~~34,~~ wherein (as claim 30).

36. An *in vivo* method of identifying compounds
that do not inhibit protoporphyrinogen oxidase
activity, comprising (a) culturing in the presence of
a test compound a sensitive microorganism, containing
a gene encoding a protein with protoporphyrinogen
oxidase activity sensitive to protoporphyrinogen
oxidase inhibitors, and a resistant microorganism,
which differs from said sensitive microorganism only
by a gene encoding a protein with protoporphyrinogen
oxidase activity resistant to protoporphyrinogen
oxidase inhibitors in which the amino acid
corresponding to Val13 of SEQ. ID. No.: 1, SEQ. ID.
No.: 2 or SEQ. ID. No.: 3 is replaced by another amino
acid, and (b) identifying the compounds which inhibit

growth of both of said sensitive and resistant microorganisms.

37. The method of identifying and evaluating a compound that does not affect protoporphyrinogen oxidase activity according to claim 36, wherein the resistant microorganism is obtained by introducing a gene encoding a protein having protoporphyrinogen oxidase activity resistant to porphyrin herbicides in which the Val13 of SEQ. ID. No.: 1, SEQ. ID. No.: 2 or SEQ. ID. No.: 3 is replaced by another amino acid in a mutant microorganism lacking active protoporphyrinogen oxidase, thereby restoring the growth ability of the mutant.

38. The method of identifying and evaluating a compound that does not affect protoporphyrinogen oxidase activity according to claim 36, wherein the resistant microorganism is obtained by introducing a gene encoding a protein having protoporphyrinogen oxidase activity resistant to porphyrin herbicides, in which the Val13 of SEQ. ID. No.: 1, SEQ. ID. No.: 2 or SEQ. ID. No.: 3 is replaced by another amino acid, into a *Chlamydomonas* strain sensitive to protoporphyrinogen oxidase-inhibiting herbicides.

39. The method of identifying and evaluating a compound that does not affect protoporphyrinogen oxidase according to claim 36 wherein said gene encoding a protein with protoporphyrinogen oxidase activity resistant to protoporphyrinogen inhibitors is a gene comprising a DNA fragment as claimed in either of claims 20 or 22.

40. The method of identifying and evaluating a compound that does not affect protoporphyrinogen oxidase activity according to ~~any one of claims 36 to~~ ^{claims 36}

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39 wherein said resistant microorganism is obtained by
introducing a gene encoding a protein having
protoporphyrinogen oxidase activity in which Val13 of
SEQ. ID. No.: 1, SEQ. ID. No.: 2 or SEQ. ID. No.: 3 is
5 replaced by Met or in which G37 of SEQ. ID. No.: 4,
SEQ. ID. No.: 5 or SEQ. ID. No.: 6 is replaced by
adenine.

add 7
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